Analysis of nanoliter volumes of antidepressants by capillary LC

Maria Serwe

Abstract

Reducing the internal diameter of an LC column to capillary dimensions increases sensitivity by decreasing the peak volume for a given injected amount. Therefore, this can provide the same sensitivity as is found in a conventional 4.6-mm column but injecting very much smaller volumes of the same concentration sample. However, to fully exploit these benefits the LC system must be able to handle sample volumes in the sub-microliter range. Here we demonstrate the ability of the Agilent 1100 Capillary LC system to handle small sample volumes accurately and reproducibly. Mixtures of tricyclic antidepressants were used as exemplary analytes. Excellent data on precision, linearity and repeatability are given for 100-nL injections on a 500-µm id capillary column.

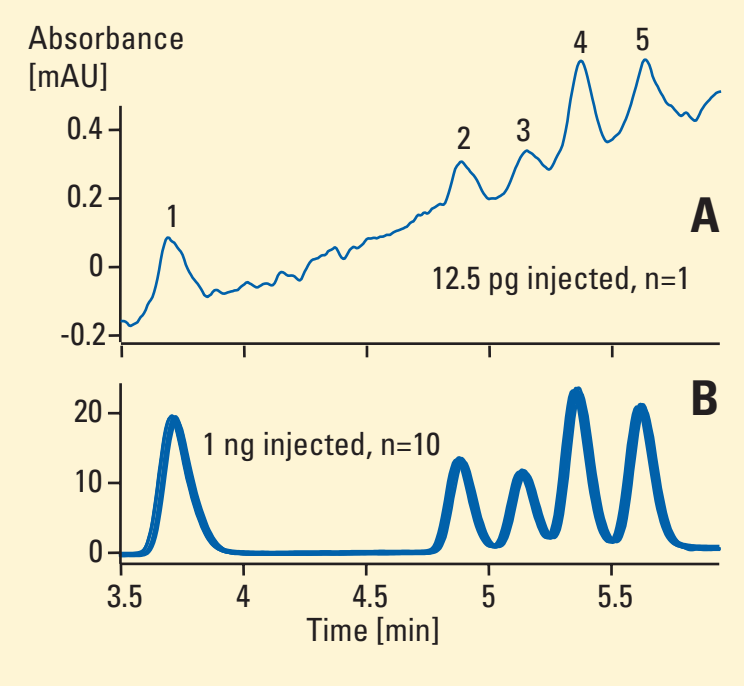

Figure 1
Analysis of a mixture of doxepin (1), desipramine (2), imipramine (3), nortriptyline (4), and amitriptyline (5)
A = analysis at the method detection limit
B = overlay of 10 runs

Conditions

Sample
standard mixture (A= 125 pg/µL, B= 10 ng/µL
Column
500 µm id, 3.5 cm length, ZORBAX SB-C18, 3.5 µm
Solvent A
25 mM NaH_2PO_4, pH 2.5 (H_2SO_4)
Solvent B
acetonitrile
Flow rate
15 µL/min
Gradient
0 min 22 % B, 5 min 35 % B, 7 min 35 % B, 8 min 22 % B, 11 min 2 % B
Column compartment temperature
40° C
Injection volume
100 nL
Detection
signal 225/10 nm, reference 450/80 nm, 500-nL volume, 10-mm path length flow cell

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Experimental

An Agilent 1100 Series Capillary LC system with Agilent ChemStation for instrument control, data acquisition and analysis was used for all experiments. The analytical column from the ZORBAX Series is also available from Agilent Technolgies. All chemicals were of HPLC grade or better. The antidepressants were purchased as HCl salts from Sigma. Multi-component samples were prepared daily from aqueous 100 mg/L stock solutions.

Results

Figure 1A shows the analysis of 5 tricyclic antidepressants near the method detection limit. An absolute injection of 12.5 pg gave signal-to-noise values of 3.3 - 8.7. In figure 1B an overlay of 10 runs at the 10 ng/µL-level is depicted. Relative standard deviation (RSD) was < 0.5 % for retention time and < 2 % for area, respectively. Analyzing a 2-ng/µL sample resulted in RSD for retention time of < 0.5 % and RSD for area of < 3 %. The assay was linear in a range from 125 pg/µL to 4 ng/µL ($r^2 > 0.999$).

Even when dealing with extremely small sample volumes the Agilent 1100 Series Capillary LC system is capable of giving highly reproducible quantitative results. This pseudo non-destructive analysis makes the system ideal in situations where sample volumes are limited and multiple analyses are necessary on a single sample.

Equipment

Agilent 1100 Capillary LC system consisting of

- Capillary pump
- Micro vacuum degasser
- Thermostatted column compartment
- Micro autosampler
- Diode array detector, 500-nL flow cell
- Agilent ChemStation

Column

- ZORBAX SB-C18, 3.5 µm (part number 5064-8260)

Maria Serwe is an application chemist at Agilent Technologies, Waldbronn, Germany

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